

## Chromosomal studies in species of *Salvia* (Lamiaceae) from Argentina

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Meiotic and mitotic chromosomes of 13 species of *Salvia* from Argentina were studied. Most of our data are new counts but some, mainly from cultivated species, confirm previous reports or represent numbers which are different from those cited previously. Only *Salvia gilliesii* Benth. and the introduced species *S. coccinea* Juss., *S. farinacea* Benth., *S. involucrata* Cav. and *S. microphylla* Kunth are diploids. *S. cardiophylla* Benth., *S. procurrens* Benth., *S. splendens* Roem. & Schult. and *S. uliginosa* Benth. are tetraploids; *S. stachydifolia* Benth. is hexaploid; and *S. guaranitica* A. St.-Hil., *S. pallida* Benth. and *S. rypara* Briq. are octoploids. The basic number most frequently found is  $x = 11$ , but two species, *S. procurrens* and *S. uliginosa*, have  $x = 13$ . *S. farinacea* has  $x = 10$  but this species is a widespread cultivated plant, not native in this area. Seven of the studied species showed one to three B chromosomes. We discuss some systematic and evolutionary aspects of the genus in the light of the cytogenetic data, the relationships between geographical distribution and chromosome numbers in relation to levels of ploidy and basic chromosome numbers, and make comparisons with some of the different taxonomic sections. © 2003 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2003, 141, 483–490.

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### INTRODUCTION

*Salvia* L. with over 900 species is probably the largest genus of the Lamiaceae (Standley & Williams, 1973). It is distributed throughout the Old and New World, in subtropical and temperate areas. The centres of diversity are the Mediterranean region, Central Asia, the highlands of Mexico and the South American Andes. The genus is subdivided into a number of groups of which subgenus *Calosphace* is exclusively New World with the South American and the majority of Central American species belonging to it (Harley & Heywood, 1992).

In Argentina there are 19 native (Xifreda, 1999) and five introduced or naturalized species of *Salvia*. The native ones are grouped in 11 of the sections proposed by Epling (1939). These taxa occur in two large disjunct areas, the north-east and the north-west of

the country. Within the framework of systematic studies on Argentine species of *Salvia*, meiotic and mitotic analyses were carried out in 13 taxa of this genus growing there: *Salvia cardiophylla* Benth., *S. coccinea* Juss., *S. farinacea* Benth., *S. gilliesii* Benth., *S. guaranitica* A. St.-Hil., *S. involucrata* Cav., *S. microphylla* Kunth, *S. pallida* Benth., *S. procurrens* Benth., *S. rypara* Briq., *S. splendens* Roem. & Schult., *S. stachydifolia* Benth. and *S. uliginosa* Benth.

*Salvia rypara* and *S. stachydifolia* (sect. *Malacophyllae* (Epling) Epling) are two closely related, morphologically similar Andean species occurring in Bolivia and the north-west Argentine provinces of Salta, Jujuy, Catamarca and Tucumán; *S. rypara* between 0 and 1000 m and *S. stachydifolia* between 1500 and 3500 m. *Salvia gilliesii* (sect. *Tomentellae* (Epling) Epling), another Andean taxon, inhabits Bolivia and Chile and extends southwards to Argentina with a wider distribution. *Salvia cardiophylla* (sect. *Rudes* (Benth.) Epling), grows in Bolivia, as well

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as in the state of Mato Grosso do Sul in Brazil, Paraguay and the north-east of Argentina.

The other native species studied in this paper are all exclusively from eastern South America. *Salvia procurrens*, a creeping herb with cordate leaves, and *S. uliginosa* (sect. *Uliginosae* (Epling) Epling), an erect one with lanceolate leaves, occupy areas of Brazil, Uruguay and Argentina. *Salvia guaranitica* (sect. *Coeruleae* Epling) and *S. pallida* (sect. *Farinaceae* (Epling) Epling) occur in Argentina, although they are also found in Brazil, Paraguay and Uruguay.

Chromosomal data on species grown in Europe, Asia and North American are abundant. However, data on South American species, which all belong exclusively to subgenus *Calosphace* (Benth.) Benth., are almost unknown. Since this region is the southernmost area of distribution, the aim of the present study is to provide karyological data for this important pool of *Salvia* species. Most of our data are new counts but some, mainly from cultivated species, confirm previous reports or represent numbers which are different from those cited previously. We also discuss some systematic and evolutionary aspects of the genus in the light of the cytogenetic data, the relationships between geographical distribution and chromosome numbers in relation to levels of ploidy and basic chromosome numbers, and make comparisons with some of the different taxonomic sections.

## MATERIAL AND METHODS

Flower buds and nutlets of native species were collected from wild populations and those of the introduced species from cultivated plants. In the case of *Salvia coccinea*, although it is native in some prov-

inces of Argentina, the material was obtained from cultivation. The species names with their sections, populations studied and status are listed in Table 1. The voucher specimens are deposited in the Herbaria SI (Instituto de Botánica Darwinion, San Isidro, Argentina) or LP (Facultad de Ciencias Naturales y Museo, La Plata, Argentina).

Ripe nutlets collected from natural populations were germinated on wet filter paper in Petri dishes. The root tip meristems were pretreated in 2 mM 8-hydroxyquinoline for 4 h, fixed in absolute ethanol and glacial acetic acid (3:1) for 4 h at room temperature and stored in the fixative at 4–5 °C. Root tips were hydrolyzed in 5 N HCl for 15 min at room temperature, and squashed in a drop of propionic acid haematoxylin (2%).

For meiotic studies, flower buds were fixed either in ethanol–chloroform–glacial acetic acid (6:3:1), or in absolute ethanol and glacial acetic acid (3:1) for at least 24 h, and then transferred into 70% ethanol and stored at 4–5 °C until required. Immature anthers were then squashed in propionic acid haematoxylin (2%) using ferric citrate as a mordant. Meiosis was studied using a minimum of 20 PMCs (pollen mother cells). Photographs were taken using a Leitz Camera. Pollen stainability was studied with Alexander's differential staining (Alexander, 1969).

## RESULTS AND DISCUSSION

The chromosome numbers ( $2n$ ), type of chromosome, basic numbers and levels of ploidy of the 13 *Salvia* species studied are presented in Table 2. For the native species all are new records except those for *S. gilliesii* and *S. guaranitica* (see Table 2 and text below).

**Table 1.** Sections, vouchers and status of the *Salvia* species studied

Species	Section	Voucher	Status
<i>S. cardiophylla</i> Benth.	<i>Rudes</i>	Entre Ríos. Dpto. Colón. <i>Sanso et al. 55</i> (SI)	Native
<i>S. coccinea</i> Juss.	<i>Subrotundae</i>	Buenos Aires. Pdo. La Plata. <i>Alberto 11</i> (LP).	Cultivated
<i>S. farinacea</i> Benth.	<i>Farinaceae</i>	Buenos Aires. Pdo. Benito Juárez. <i>Sanso 75</i> (SI)	Cultivated
<i>S. gilliesii</i> Benth.	<i>Tomentellae</i>	Tucumán. Dpto. Tafí. <i>Xifreda, Sanso &amp; Mallo 2090</i> (SI)	Native
<i>S. guaranitica</i> A. St.-Hil.	<i>Coeruleae</i>	Buenos Aires. Pdo. La Plata. <i>Xifreda &amp; Alberto 2050</i> (SI)	Native
<i>S. involucrata</i> Cav.	<i>Cardinales</i>	Buenos Aires. Pdo. Vicente López. <i>Hunziker 13274</i> (SI)	Cultivated
<i>S. microphylla</i> Kunth	<i>Fulgentes</i>	Buenos Aires. Pdo. Benito Juárez. <i>Sanso 52</i> (SI)	Cultivated
<i>S. pallida</i> Benth.	<i>Farinaceae</i>	Entre Ríos. Dpto. Ceibas. <i>Wulff 813</i> (LP)	Native
<i>S. procurrens</i> Benth.	<i>Uliginosae</i>	Buenos Aires. Pdo. Ensenada. <i>Xifreda &amp; Alberto 3008</i> (LP)	Native
<i>S. rypara</i> Briq.	<i>Malacophyllae</i>	Salta. Dpto. Capital. <i>Xifreda, Sanso &amp; Mallo 2081, 2084</i> (SI)	Native
<i>S. splendens</i> Roem. & Schult.	<i>Secundae</i>	Buenos Aires. Pdo. La Plata. <i>Alberto 12</i> (LP)	Cultivated
<i>S. stachydifolia</i> Benth.	<i>Malacophyllae</i>	Tucumán. Dpto. Tafí. <i>Xifreda, Sanso &amp; Mallo 2095</i> (SI)	Native
<i>S. uliginosa</i> Benth.	<i>Uliginosae</i>	Entre Ríos. Dpto. Gualeguaychú. <i>Alberto &amp; Lo Gioco 7</i> (LP)	Native

**Table 2.** Chromosome numbers ( $2n$ ), type of chromosomes, basic numbers and levels of ploidy of the *Salvia* species studied

Species	Chromosome number ( $2n$ )	Chromosomes studied	Basic number ( $x$ )	Ploidy level
<i>S. cardiophylla</i> Benth.	44 + 0–1B*	meiotic	11	4x
<i>S. coccinea</i> Juss.	22 + 0–1B	meiotic	11	2x
<i>S. farinacea</i> Benth.	20 + 0–1B	meiotic	10	2x
<i>S. gilliesii</i> Benth.	22	mitotic	11	2x
<i>S. guaranitica</i> A. St.-Hil.	88	meiotic/mitotic	11	8x
<i>S. involucrata</i> Cav.	22 + 0–1B	meiotic	11	2x
<i>S. microphylla</i> Kunth	22	meiotic	11	2x
<i>S. pallida</i> Benth.	88*	meiotic	11	8x
<i>S. procurrens</i> Benth.	52 + 0–1B*	meiotic	13	4x
<i>S. rypara</i> Briq.	88*	meiotic/mitotic	11	8x
<i>S. splendens</i> Roem. & Schult.	44 + 0–1B	meiotic	11	4x
<i>S. stachydifolia</i> Benth.	66*	meiotic	11	6x
<i>S. uliginosa</i> Benth.	52 + 0–3 Bs*	meiotic	13	4x

\*New records.

Only *Salvia gilliesii* and the introduced or naturalized species *S. coccinea*, *S. farinacea*, *S. involucrata* and *S. microphylla* are diploids. *Salvia cardiophylla*, *S. procurrens*, *S. splendens* and *S. uliginosa* are tetraploids. *Salvia stachydifolia* is hexaploid and *S. guaranitica*, *S. pallida* and *S. rypara* are octoploids.

The basic number most frequently found is  $x = 11$ , but two species, *S. procurrens* and *S. uliginosa*, presented  $x = 13$ . *Salvia farinacea* has  $x = 10$  but this species is a widespread cultivated taxon, not native in Argentina. Seven of the studied species showed one to three B chromosomes (Table 2).

#### *SALVIA CARDIOPHYLLA* BENTH.

Diakinesis (Figs 3,17B) and metaphase I showed 22 II + 0–1 B chromosome.

#### *SALVIA COCCINEA* JUSS.

Diakinesis and metaphase I showed 11 II + 0–1 B chromosome. It agrees with the many chromosome counts that have been published for this widespread species introduced or naturalized in subtropical regions of both hemispheres (Harley & Heywood, 1992 and previous reports cited by these authors).

#### *SALVIA FARINACEA* BENTH.

The meiotic configurations observed in this ornamental plant, native in Texas and New Mexico (Epling, 1939), were 10 II or 8 II + 1 IV (Figs 15,17I). The quadrivalent (Fig. 16) can adopt different morpholo-

gies at diakinesis and metaphase I. In some cells one B chromosome could be seen (Fig. 15). The individuals studied seem to be heterozygous for one reciprocal translocation.  $2n = 20$  has been cited before (Peterson, 1978, in Harley & Heywood, 1992), but a different number,  $2n = 18$  was also reported by Bir *et al.* (1978), Haque & Ghoshal (1980) and Haque (1981).

#### *SALVIA GILLIESII* BENTH.

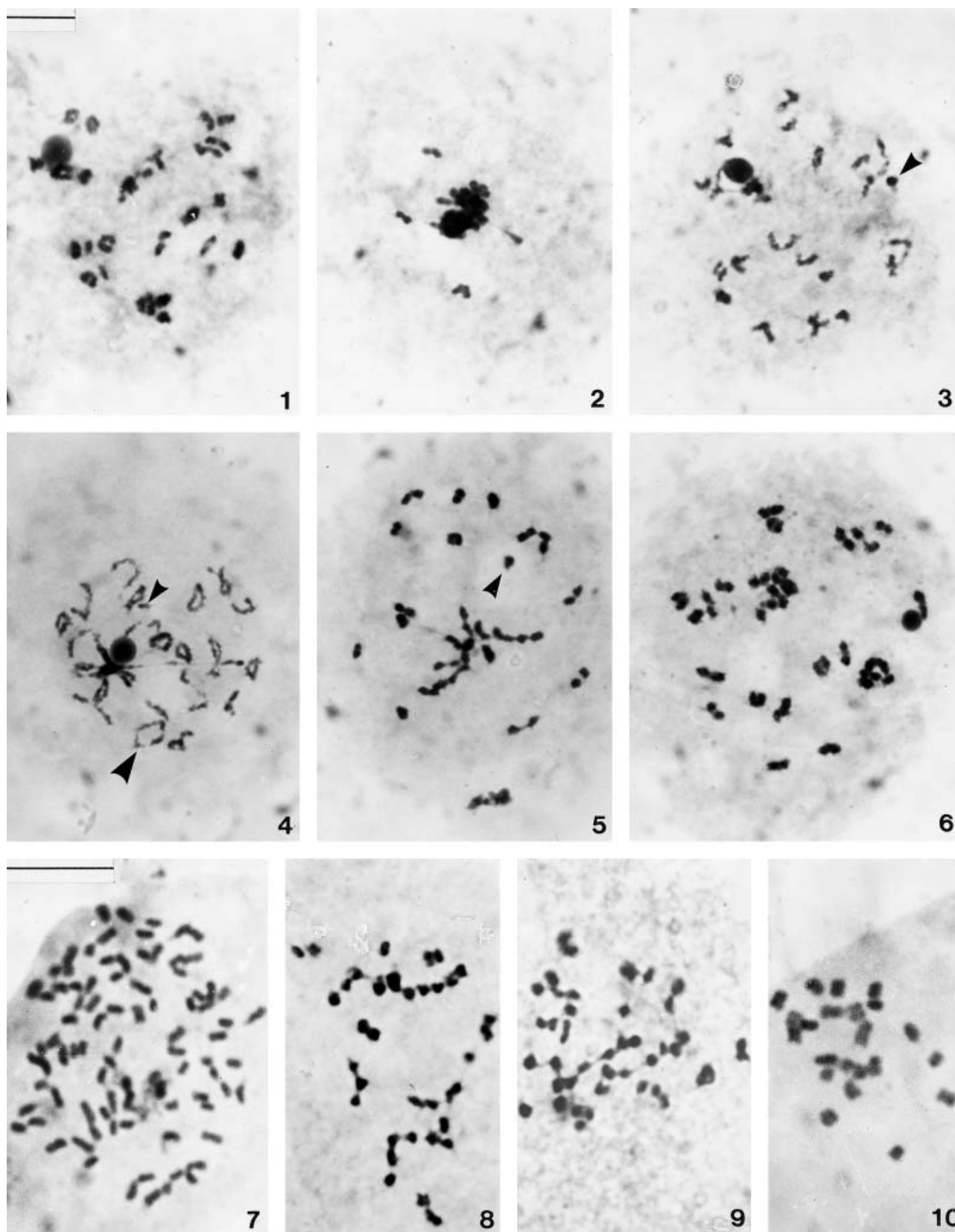
The number  $2n = 22$  confirms a previous finding obtained from a meiotic study of this species (Bernardello, Stiefkens & Piovano, 1990). Twenty-two somatic chromosomes were observed at metaphase (Fig. 10).

#### *SALVIA GUARANITICA* A. ST.-HIL.

Our data,  $2n = 88$ , obtained from meiotic and mitotic studies, do not agree with the previous report of  $2n = 84$  (Harley & Heywood, 1992). Forty-four bivalents were observed at diakinesis and 88 chromosomes at mitotic metaphase (Figs 7,17E).

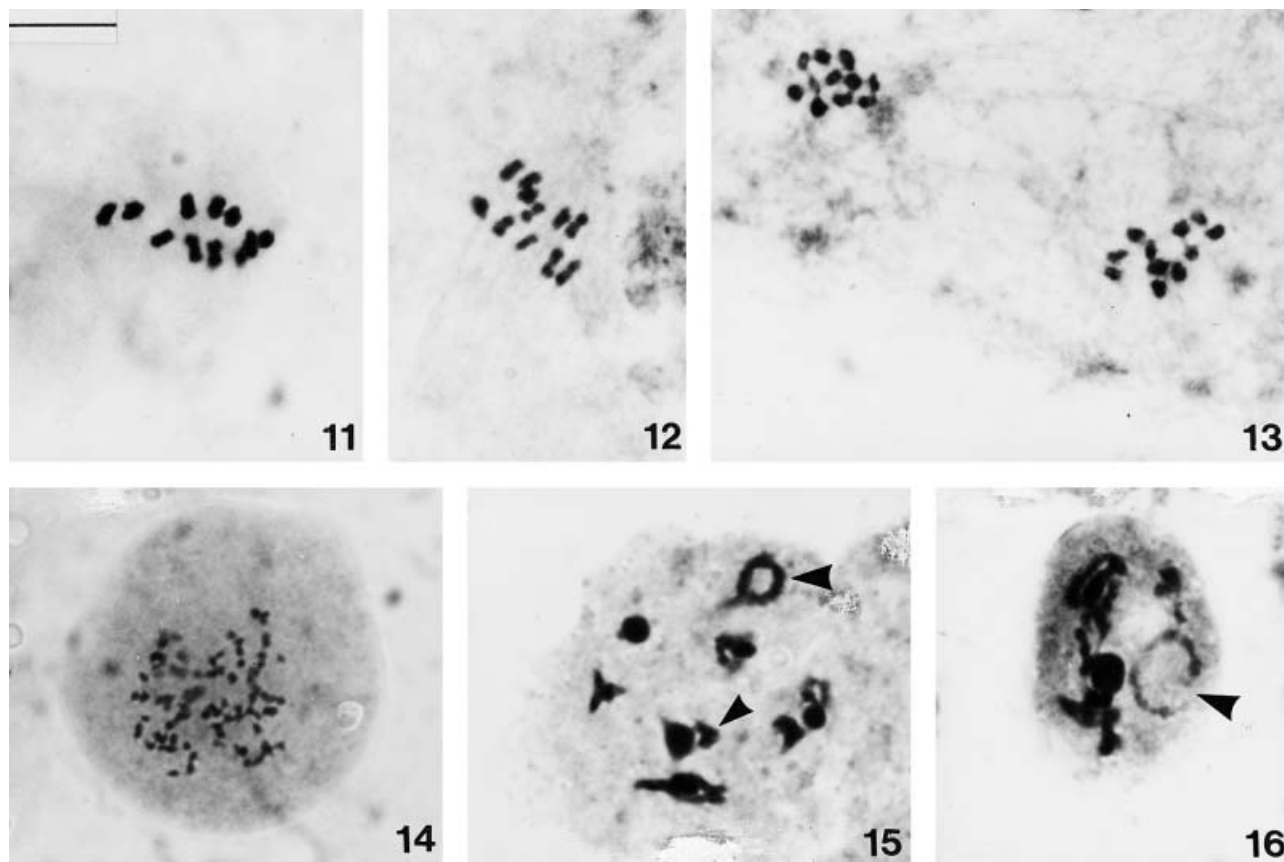
#### *SALVIA INVOLUCRATA* CAV.

In this species, native in Mexico, meiotic chromosome counts showed  $2n = 22$  chromosomes. Eleven bivalents at diakinesis and metaphase I (Figs 11,12) and 11 chromosomes at metaphase II were observed (Fig. 13). However,  $n = 7$  was cited by Gill (1984). The presence of one B chromosome is reported here for the first time.





**Figures 1–10.** Meiotic and mitotic chromosomes. Figs 1,2. *Salvia uliginosa* (Alberto & Lo Gioco 7). Fig. 1. Diakinesis, 26 II. Fig. 2. Metaphase I with I and II non-congressed. Fig. 3. *S. cardiophylla* (Sanzo et al. 55), diakinesis, 22 II + 1 B chromosome (arrowhead). Figs 4–6. *S. procurrens* (Xifreda & Alberto 3008). Fig. 4. Diplotene (large arrowhead: IV; small arrowhead: I, B chromosome). Figs 5,6. Diakinesis. Fig. 5. 26 II + 1 B chromosome (arrowhead), some bivalents with sticky connections. Fig. 6. 26 II. Fig. 7. *Salvia guaranitica* (Xifreda & Alberto 2050), mitotic metaphase,  $2n = 88$  chromosomes. Fig. 8. *S. stachydifolia* (Xifreda, Sanzo & Mallo 2095), diakinesis, 33 II, some bivalents with sticky connections. Fig. 9. *S. rypara* (Xifreda, Sanzo & Mallo 2081), diakinesis, 44 II, some bivalents with sticky connections. Fig. 10. *S. gilliesii* (Xifreda, Sanzo & Mallo 2090), mitotic metaphase,  $2n = 22$  chromosomes. Scale bars = 10  $\mu\text{m}$ . Figs 1–6 and 7–10 are at two different enlargements.



**Figures 11–16.** Meiotic chromosomes. Figs 11–13. *Salvia involucrata* (Hunziker 13274). Figs 11,12. Metaphase I, 11 II. Fig. 13. Metaphase II with 11 chromosomes in each group. Fig. 14. *S. pallida* (Wulff 813), diakinesis, 44 II. Figs 15,16. *S. farinacea* (Sanzo 75). Fig. 15. 8 II + 1 IV (large arrowhead) + 1 B chromosome (small arrowhead). Fig. 16. 1 IV is clearly seen (arrowhead). Scale bar = 10  $\mu\text{m}$ .

#### *SALVIA MICROPHYLLA* KUNTH

The observed chromosome number  $2n = 22$  agrees with some previous reports for this familiar garden plant native in Mexico (Haque & Ghoshal, 1980) but not with that of  $2n = 46$  by Harley & Heywood (1992).

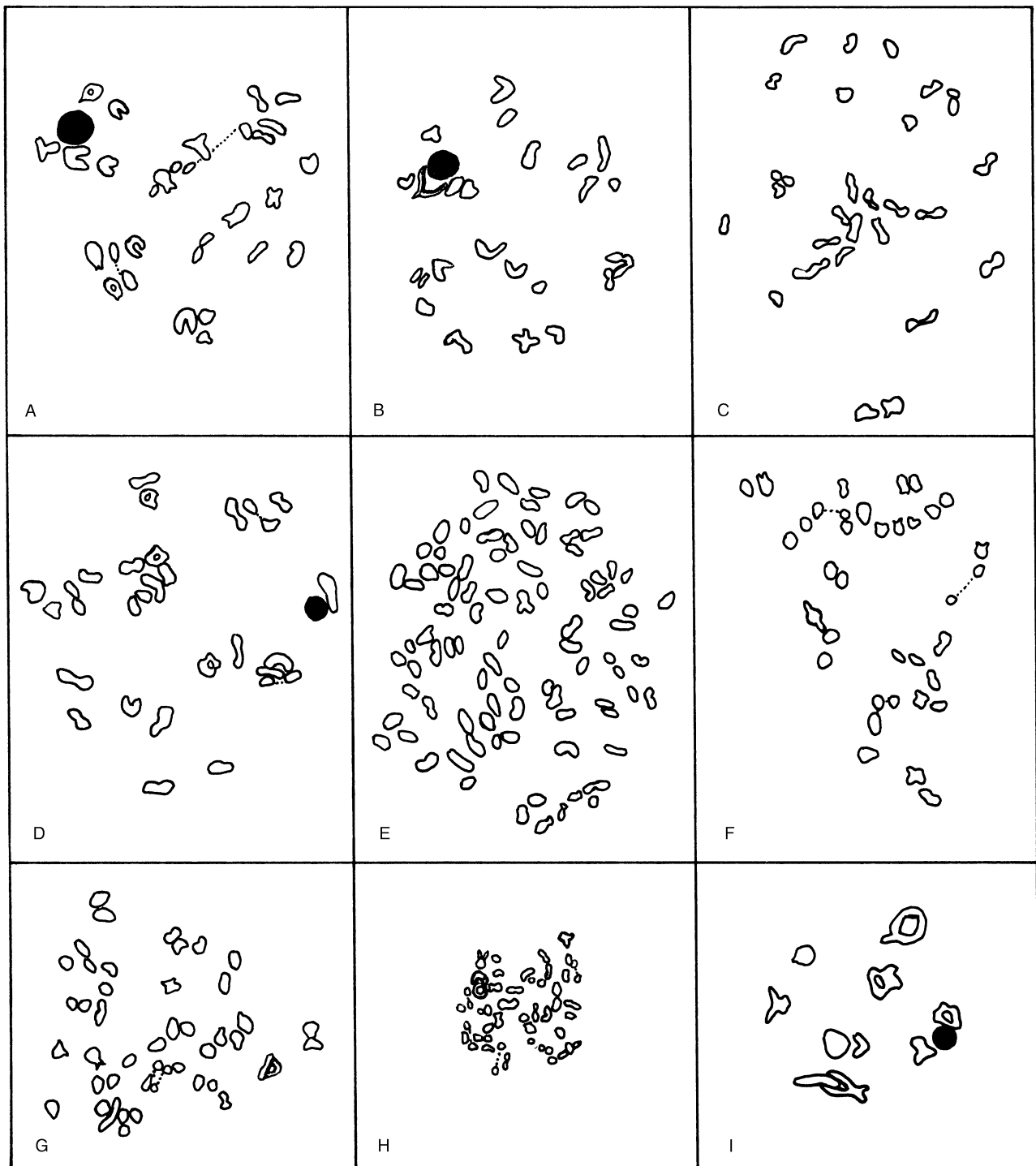
#### *SALVIA PALLIDA* BENTH.

Meiotic studies revealed 44 bivalents at diakinesis (Figs 14,17H) and metaphase I. In some cells, lagging

chromosomes were observed at anaphase I and telophase I.

#### *SALVIA PROCURRENS* BENTH.

In this species PMCs showed 26 II at diplotene and diakinesis (Fig. 5,6,17C,D), but occasionally 24 II + 1 IV could be observed (Fig. 4). Additionally, in some cells one B chromosome was visible (Figs 4,5).



**Figure 17.** Interpretation of the cells shown in Figs 1,3,5–9,14,15. (A) Cell of Fig. 1, 26 II. (B) Cell of Fig. 3, 22 II + 1 B chromosome. (C) Cell of Fig. 5, 26 II + 1 B chromosome. (D) Cell of Fig. 6, 26 II. (E) Cell of Fig. 7,  $2n = 88$  chromosomes. (F) Cell of Fig. 8, 33 II. (G). Cell of Fig. 9, 44 II. (H) Cell of Fig. 14, 44 II. (I) Cell of Fig. 15, 8 II + 1 IV + 1 B chromosome. Shaded areas represent nucleoli. Drawn at the same magnification and with the same orientation as the photographs.

*SALVIA RYPARA* BRIQ.

Counts at meiosis (Figs 9,17G) and mitosis showed a chromosome number  $2n = 88$ .

*SALVIA SPLENDENS* ROEM. & SCHULT.

Metaphase I and diakinesis exhibited 22 bivalents.  $2n = 44$  has been reported in several other papers for this species native in Brazil, but nowadays widely distributed as an ornamental (Thoppil & José, 1995; papers cited by Harley & Heywood, 1992: 243). In some cells one B chromosome could be observed.

*SALVIA STACHYDIFOLIA* BENTH.

This species was the only one found to be hexaploid. Thirty-three bivalents were seen at diakinesis (Figs 8,17F).

*SALVIA ULIGINOSA* BENTH.

As in *S. procurrens*, PMCs of this taxon showed 26 bivalents at diakinesis (Figs 1,17A), but occasionally 24 II + 1 IV could be seen. One to three B chromosomes were present. However, abnormalities were observed, such as chromosomes and bivalents non-congressed at metaphase I (Fig. 2).

## POLLEN STAINABILITY

The degree of pollen grain stainability in the species ranged from 75.7% in *Salvia guaranitica* to 97.6% in *S. gilliesii* (Table 3). Pollen stainability in *Salvia stachydifolia* could not be evaluated because of lack of pollen grains. Some of the lowest values obtained belong to the cultivated species. *Salvia guaranitica*,

although native in north-east Argentina, can be found as a cultivated plant in other areas of the country.

## CONCLUSIONS

*Salvia* has more than one basic chromosome number. Studies carried out on subgenus *Calosphace* indicate that the one most frequently found is  $x = 11$ . Some taxa have basic chromosome numbers lower than 11,  $x = 6, 7$  and 10 (reports from Harley & Heywood, 1992 and previous ones cited by these authors). However, this is the first time that  $x = 13$  has been obtained in this subgenus. This last number,  $x = 13$ , is a dibasic number, arising from  $x = 6$  and  $x = 7$  in combination. The same number is also present in *S. columbariae* Benth., section *Pycnosphace*, *Salvia* subg. *Audibertia* (Epling, Lewis & Raven, 1962).

The species from north-west Argentina all have  $x = 11$ , but with different levels of ploidy. The ones from the north-east have at least two different basic numbers,  $x = 11$  and  $x = 13$ . Until now, the only species with  $x = 13$ , *S. procurrens* and *S. uliginosa*, are polyploids and belong to the same taxonomic group, section *Uliginosae* (Epling) Epling. The first record of a hexaploid species in *Salvia* subgenus *Calosphace* was given for a Mexican taxon, *S. lavanduloides* Kunth (Palomino, Mercado & Ramamoorthy, 1986). *Salvia stachydifolia* (this paper) is thus the second case of hexaploidy. Plant habit would not seem to relate to the level of ploidy, since two of the species investigated in the present study which are shrubby, *S. gilliesii* and *S. guaranitica*, are diploid and octoploid, respectively.

B chromosomes, observed here in several of the species studied (*S. cardiophylla*, *S. coccinea*, *S. farinacea*, *S. involucrata*, *S. procurrens*, *S. splendens* and *S. uliginosa*), have been recorded before in the genus (Afzal-Rafh, 1976; Mercado, Ramamoorthy & Palomino, 1989).

The data obtained for two of the cultivated species, *S. coccinea* and *S. splendens*, agree in some cases with data obtained from material collected outside Argentina.

The sections of *Salvia* subgenus *Calosphace* established by Epling (1939) require a careful review. They do not seem to represent natural groups. Their systematic and taxonomic significance needs re-evaluation, since at least some of them are undoubtedly polyphyletic. An example of this is the section *Farinaceae* (Epling) Epling, which groups species from USA and Mexico with basic chromosome numbers  $x = 6, 7$  and 10 (Harley & Heywood, 1992: 242; *S. farinacea*, this paper) and a single one from South America, *S. pallida*, with  $x = 11$ . We consider that *S. pallida* should be excluded from this section because of its distribution distant from the rest of the

**Table 3.** Pollen stainability of the *Salvia* species studied

Species	% stainability
<i>S. cardiophylla</i>	93.85
<i>S. coccinea</i>	77.87
<i>S. farinacea</i>	92.31
<i>S. gilliesii</i>	97.60
<i>S. guaranitica</i>	75.70
<i>S. involucrata</i>	87.35
<i>S. microphylla</i>	88.74
<i>S. pallida</i>	84.01
<i>S. procurrens</i>	96.98
<i>S. rypara</i>	94.00
<i>S. splendens</i>	79.25
<i>S. stachydifolia</i>	—
<i>S. uliginosa</i>	91.81

species and the uniqueness of its basic chromosome number.

With the probable centre of origin for *Calosphace* in Central America there must have been a secondary spread to the Andes, where polyploidy is apparently more developed. Eastern South America appears to have been the last area to be colonized, with a further increase in ploidy level (Harley & Heywood, 1992). The new chromosome data for these South American species showed only polyploid species, with the exception of *S. gilliesii*, and revealed high levels of ploidy. Previous data from that area, although very scattered, also indicate only the presence of polyploids: *S. greuiifolia* S. Moore (sect. *Hoehnea*),  $2n = 44$ ; *S. itatiaiensis* Dusén (sect. *Angulatae*),  $2n = 84$ ; *S. ombrophila* Dusén (sect. *Tubiflorae*),  $2n = 240$ ; *S. tomentella* (sect. *Albolanatae*),  $2n = 86$  (Harley & Heywood, 1992). Wider sampling of South American species is needed to understand how the evolution has proceeded in the group but, obviously from the results obtained in this paper, it is evident that polyploidy is at least one of the evolutionary strategies of species of *Salvia* in this area.

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